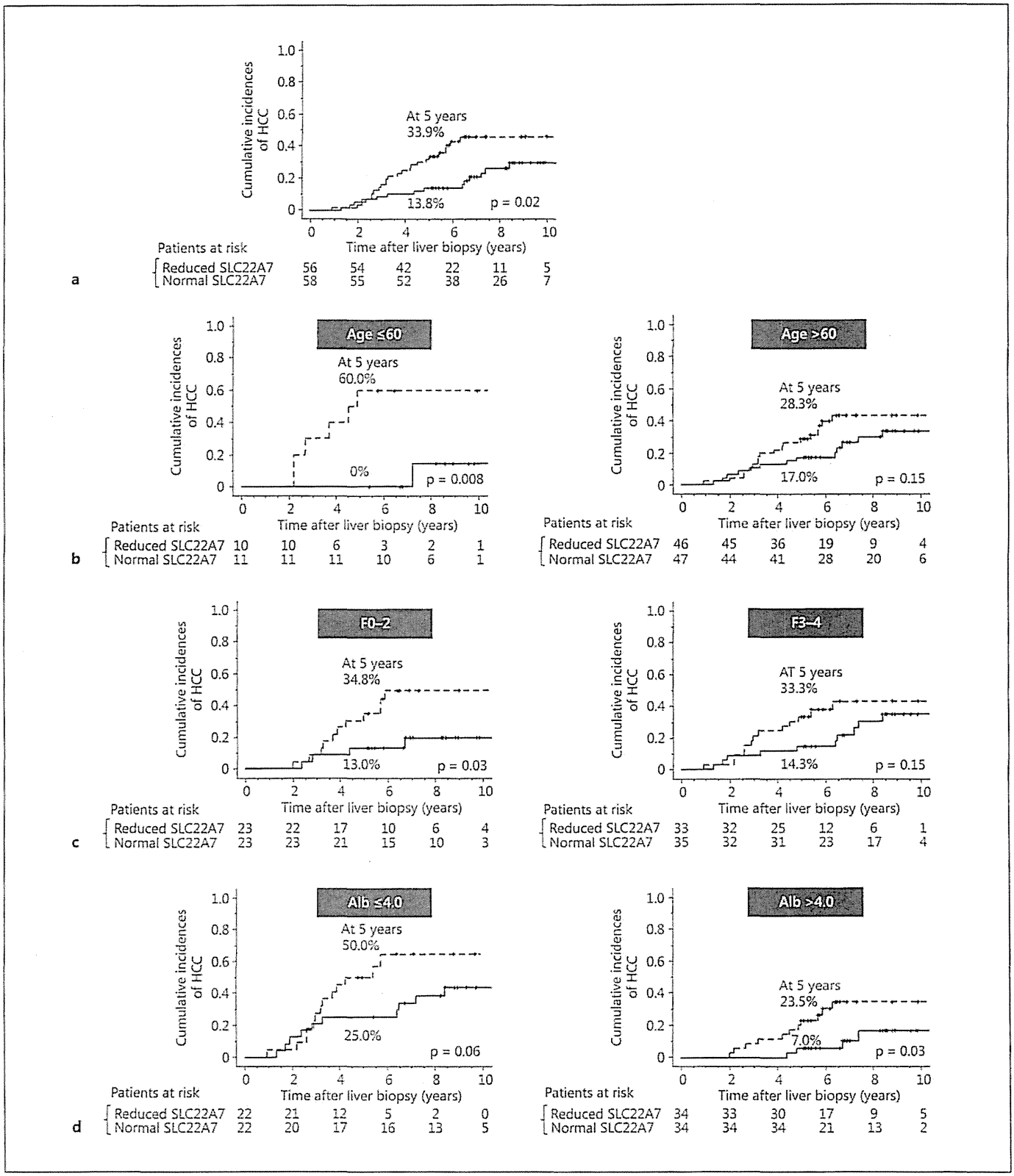

Fig. 6. Cumulative incidence of HCC according to SLC22A7 staining. **a** Comparison of the cumulative incidences of HCC in patients with normal (solid line) and reduced SLC22A7 expression (broken line). **b–d** The cumulative incidences of HCC after stratification by age (**b**), fibrosis stage (**c**) and albumin (Alb) level (**d**), respectively.

tients without advanced fibrosis, SLC22A7 expression can provide an important cost-effective screening tool. Moreover, we confirmed previous knowledge of low serum albumin levels as an independent risk factor for HCC development in patients matched for age, gender and stage of liver fibrosis. Nonetheless, in patients with higher serum albumin levels (≥4.0 g/dl), reduced SLC22A7 expression remained a significant independent risk factor for HCC.

The *SLC22A7* gene encodes OAT2, which is distributed mainly in the liver and kidney. As a protein predominantly expressed in the liver [23], OAT2 transports several antiviral drugs as well as prostaglandins. A recent study in rats showed that OAT2 is responsible for the uptake of orotic acid [24], which reportedly promotes liver carcinogenesis [25, 26]. In the clinical setting, orotic aciduria was also observed in HCC patients without liver cirrhosis [27]. Moreover, a previous study using gene-set enrichment analysis revealed that SLC22A7 expression is significantly correlated with mitochondrial oxidoreductase activity and fatty acid metabolism. Mitochondrial dysfunction and oxidative stress are considered key mechanisms for the development of HCC. Collectively, these studies indicate that reduced SLC22A7 expression promotes hepatic carcinogenesis by increasing the concentration of orotic acid around hepatocytes and promoting oxidative stress and mitochondrial dysfunction. Our study suggests that these microenvironmental changes might occur in patients with chronic HCV in an early stage. As for HCC recurrence after surgical resection, gene expression has been extensively investigated in tissues surrounding HCC [16, 28–30]. However, it remains unknown whether these signatures correlate with multifocal occurrence of HCC. Indeed, the precise mechanisms involved in the association between SLC22A7 expression and HCC development require further investigation.

In this study, personally gifted antibody was used for IHC. Staining performance of our antibody was similar to that of commercially available antibodies (Atlas Antibodies, Stockholm, Sweden) by a small pilot study (unpubl. data).

Our retrospective study design and low patient numbers must be acknowledged as limitations, particularly in the first study. However, this first study confirmed that our biopsy specimens were feasible for IHC analysis of SLC22A7, and we could therefore proceed to the larger matched-control study. To improve reproducibility, we conducted a propensity score matched study and only included patients who were HCV-positive and had not achieved SVR with interferon therapy, so our results may not pertain to chronic HCV patients who achieve SVR or patients with other chronic diseases of the liver. A larger prospective study will be required to confirm our results.

In conclusion, our study showed the importance of IHC staining for SLC22A7 as a predictive tool for HCC. We propose that patients with reduced SLC22A7 expression and lower serum albumin levels are candidates for intensive HCC surveillance, even if they do not exhibit other known risk factors.

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Clinical Efficacy of Nontransplant Therapies in Patients with Hepatocellular Carcinoma with Child-Pugh C Liver Cirrhosis

HIROKI NISHIKAWA, RYUICHI KITA, TORU KIMURA, YOSHIAKI OHARA, HARUHIKO TAKEDA, AZUSA SAKAMOTO, SUMIO SAITO, NORIHIRO NISHIJIMA, AKIHIRO NASU, HIDEYUKI KOMEKADO and YUKIO OSAKI

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Abstract. *Aim: To compare clinical outcome in patients with Child-Pugh C hepatocellular carcinoma (HCC) treated with nontransplant therapies and those treated with best supportive care. Patients and Methods: A total of 182 patients with HCC with Child-Pugh C cirrhosis were analyzed. Patients were classified into two groups: patients treated with nontransplant therapies (n=113, treated group) and untreated patients (n=69, untreated group). Furthermore, for reducing the bias in patient selection, a propensity score matching analysis was performed (55 pairs). Results: The median survival time in the treated group was significantly longer than that in the untreated group (1.16 years vs. 0.21 years, p<0.001). After propensity score matching, the median survival time in the treated group remained significantly longer than that in the untreated group (0.95 years vs. 0.17 years, p=0.010). Conclusion: In patients with HCC with Child-Pugh C cirrhosis, those treated with nontransplant therapies might have longer survival than untreated patients.*

Hepatocellular carcinoma (HCC) is a major health problem worldwide. It is the fifth most common cancer in men and the seventh in women and the third most common cause of cancer-related death (1-4). In Japan, as well as in other countries, most cases of HCC are associated with viral infections such as hepatitis B (HBV) and hepatitis C (HCV) virus, although in our country, the number of patients with HCC with etiologies other than HBV and HCV has recently been increasing (5, 6). The prognosis for untreated HCC is poor in general, and the curative treatments for this disease comprise surgical resection, ablative therapies such as radiofrequency ablation and percutaneous ethanol injection, and liver transplantation (1-6). Noncurative therapies for HCC include transcatheter arterial chemoembolization (TACE), transcatheter arterial infusion chemotherapy, radioembolization, molecular targeting therapies such as sorafenib, and radiation therapy (1-10).

Prognosis for HCC in patients with Child-Pugh C cirrhosis is extremely poor. Thus, in these patients, most current HCC practice guidelines recommend liver transplantation for patients within Milan criteria and best supportive care for patients without Milan criteria (1, 4, 11, 12). However, in Japan, due to the limited number of brain death donors and advanced age of patients with HCC, the Japan Society of Hepatology recommends nontransplant therapies such as transcatheter arterial chemotherapy with or without embolization and ablative therapies even in HCC with Child-Pugh C cirrhosis (13). The number of elderly patients with HCC in our country has been increasing in recent years (14). However, whether patients with HCC with Child-Pugh C cirrhosis treated with nontransplant therapies could obtain survival benefit remains unclear.

The aims of the current study were thus to compare clinical outcome in patients with HCC with Child-Pugh C cirrhosis treated with nontransplant therapies and those treated with best supportive care. Furthermore, for reducing the bias in patient selection, we compared clinical outcome of these two groups using propensity score matching analysis.

Patients and Methods

Patients. Between May 1990 and October 2013, 190 consecutive patients were initially diagnosed as having HCC with Child-Pugh C cirrhosis at the Department of Gastroenterology and Hepatology, Osaka Red Cross Hospital, Japan. Of these, seven patients with ruptured HCC and one patient within Milan criteria who underwent liver transplantation were excluded from the current analysis. A total of 182 patients were thus analyzed in the present study. Patients

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Key Words: Hepatocellular carcinoma, Child-Pugh C, Overall survival, Nontranspalnt therapies, Best supportive care.

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were classified into two groups: patients treated with nontransplant therapies (n=113, the treated group) and untreated patients (n=69, the untreated group). Since there is no clear evidence that nontransplant therapies for HCC improves the survival of patients with Child-Pugh C cirrhosis, all the patients were informed that the potential treatment benefits were unclear and the rates of expected treatment related complications were higher than those in patients with well-preserved liver function. After full explanation of HCC therapy to these patients, whether therapy for HCC was performed was mainly determined by the decision of attending physicians considering tumor burden, performance status and liver functional reserve. In the treated group, transcatheter arterial therapies were performed in 69 patients, percutaneous ablative therapies in 43 patients and surgical resection in one patient as an initial therapy for HCC. Transcatheter arterial therapies were performed with the utmost care. In other words, they were performed supraselectively in the most peripheral accessible feeding arteries to avoid irreversible liver failure. In patients with main portal vein tumor thrombus, TACE was not chosen. Overall survival (OS) was compared between the two groups.

All the protocols were approved by the ethics committee of our institution (approval number, 433). Written informed consent was obtained from all patients prior to each treatment, and the study protocol complied with all of the provisions of the Declaration of Helsinki. The present study comprised a retrospective analysis of patient records registered in our database, and all treatments were conducted in an open-label manner.

HCC diagnosis. HCC was diagnosed using abdominal ultrasound and dynamic computed tomographic (CT) scans (hyperattenuation during the arterial phase in all or some part of the tumor and hypoattenuation in the portal-venous phase) and/or magnetic resonance imaging (MRI), based mainly on the recommendations of the American Association for the Study of Liver Diseases (15-18). Arterial- and portal-phase dynamic CT images were obtained at approximately 30 and 120 s, respectively, after the injection of the contrast material. HCC stage was determined using the Liver Cancer Study Group of Japan staging system (19).

Follow-up. Follow-up consisted of periodic blood tests and monitoring of tumor markers, including α-fetoprotein (AFP) and *des*-γ-carboxy prothrombin (DCP), using chemiluminescent enzyme immunoassays (Lumipulse PIVKAII Eisai; Eisai, Tokyo, Japan). Dynamic CT/MRI scans were obtained every 2-4 months. Retreatment for HCC was considered depending on the patient's general conditions, tumor stage and background liver function.

Propensity score analysis. For reducing the bias in patient selection, a propensity score matching analysis was performed to examine causal relationships between treatments and clinical outcomes in a retrospective study other than a randomized controlled trial. Clinical variables entered into the propensity model were age, gender, Child-Pugh score, HCC stage, Milan criteria, aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Subsequently, a one-to-one match between the treated group and the untreated group was obtained by using the nearest-neighbor matching method (20, 21).

Statistical analysis. Data were analyzed using univariate and multivariate analyses. Continuous variables were compared using unpaired *t*-tests and categorical variables were compared using Fisher's exact tests. For analysis of OS, follow-up ended at the time of death from any cause, and the remaining patients were censored at the last follow-up visit. The cumulative OS rates were calculated using the Kaplan-Meier method, and tested using the log-rank test. Factors with a *p*-value <0.05 in univariate analysis were subjected to multivariate analysis using the Cox proportional hazards model. These statistical methods were used to estimate the interval from initial HCC diagnosis. Data were analyzed using SPSS for Windows (SPSS Inc., Chicago, IL, USA). Data are expressed as means±standard deviation (SD). Values of $p<0.05$ were considered to be statistically significant.

Table I. *Baseline characteristics of the treated group and the untreated group. Data are expressed as number or mean±standard deviation.*

Variable	Treated group (n=113)	Untreated group (n=69)	*p*-Value
Age (years)	64.4±7.8	64.8±10.0	0.759[a]
Gender, male/female	72/41	46/23	0.750[b]
Cause of liver disease B/C/B and C/non B non C/unknown	15/70/3/22/3	18/38/1/12/0	0.162[b]
HCC stage Stage I or II/III or IV	51/62	18/51	0.012[b]
Milan criteria Within/without	61/52	18/51	<0.001[b]
Maximum tumor size (cm)	3.8±2.6	5.5±3.6	0.001[a]
Child-Pugh score 10/11/12/13/14/15	71/28/8/3/3/0	26/25/7/7/3/1	0.010[b]
AST (IU/l)	85.9±49.8	134.2±133.3	0.001[a]
ALT (IU/l)	48.7±28.6	71.4±100.7	0.025[a]
Platelets ($\times 10^4/mm^3$)	9.3±6.4	12.5±9.3	0.012[a]
AFP (ng/ml)	20482±98379	17403±70627	0.821[a]
DCP (mAU/ml)	14216±37833	9651±25623	0.428[a]

HCC: Hepatocellular carcinoma, AST: aspartate aminotransferase, ALT: alanine aminotransferase, AFP: alpha-fetoprotein, DCP: *des*-γ-carboxy prothrombin, a: unpaired *t*-test, b: Fisher,s exact test.

Results

Baseline characteristics. The baseline characteristics of the patients in the two groups are shown in Table I. The median observation period was 0.96 years in the treated group and 0.17 years in the untreated group. In terms of maximum tumor size ($p=0.001$), AST value ($p=0.001$), ALT value ($p=0.025$) and platelet count ($p=0.012$), significant differences were observed in the two groups. The proportions of patients with stage I or II HCC ($p=0.012$), those with HCC within Milan criteria ($p<0.001$) and those with lower Child-Pugh score ($p=0.010$) in the treated group were significantly higher than those in the untreated group, suggesting that the treated group patients had less advanced HCC and better liver functional reserve than the untreated group of patients.

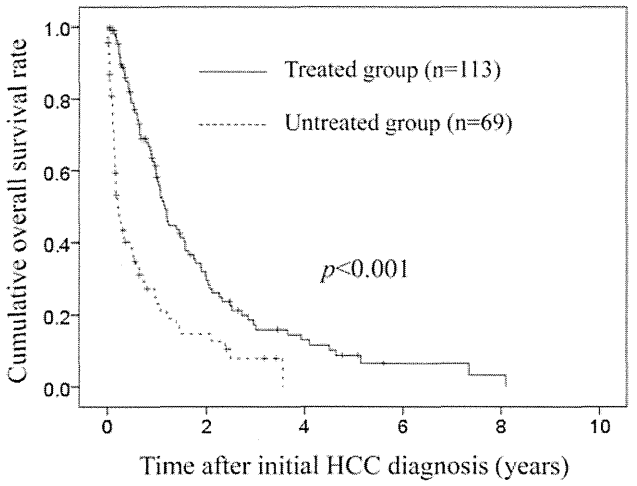

Figure 1. *Overall survival in the treated group and the untreated group. The median survival time (MST) in the treated group [MST=1.16 years, 95% confidence interval (CI)=0.98-1.34 years] was significantly longer than that in the untreated group (MST=0.21 years, 95% CI=0.11-0.31 years) (p<0.001).*

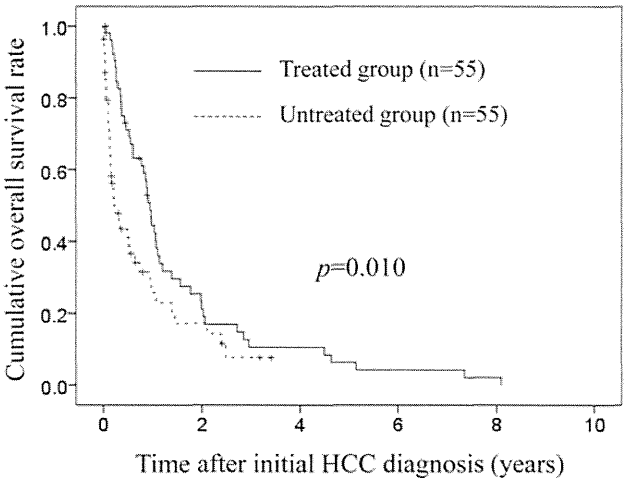

Figure 2. *Overall survival in the treated group and the untreated group after propensity score matching. The median survival time (MST) in the treated group (MST=0.95 years, 95% CI=0.82-1.08 years) was significantly longer than that in the untreated group (MST= 0.17 years, 95% CI=0.05-0.39 years) (p=0.010).*

OS in the two groups. The median survival time (MST) in the treated group [MST=1.16 years, 95% confidence interval (CI)=0.98-1.34 years] was significantly longer than that in the untreated group (MST=0.21 years, 95% CI=0.11-0.31 years) ($p<0.001$) (Figure 1).

Univariate and multivariate analyses of factors contributing to OS. Univariate analysis identified the following factors as being significantly associated with OS for all cases (n=182): treatment for HCC ($p<0.001$); Child-Pugh score 10 or 11 ($p=0.002$); HCC stage I or II ($p<0.001$); Milan criteria ($p<0.001$); maximum tumor size >3 cm ($p<0.001$); AST >80 IU/l ($p=0.003$); AFP ≥100 ng/ml ($p=0.001$); and DCP ≥300 mAU/ml ($p<0.001$) (Table II). The hazard ratios (HRs) and 95% CIs calculated using multivariate analysis for the eight factors that were significant in univariate analysis are detailed in Table II. Treatment for HCC ($p=0.003$), Child-Pugh score 10 or 11 ($p=0.006$), AFP >100 ng/ml ($p=0.004$) and DCP ≥300 mAU/ml ($p<0.001$) were found to be significant predictors linked to OS in multivariate analysis.

Causes of death in the two groups. During the follow-up period, 86 patients (76.1%) died in the treated group. The causes of death in the treated group were HCC progression in 25 patients, liver failure in 55, and miscellaneous causes in six. In the untreated group, 56 patients (81.2%) died during the follow-up period. The causes of death in the untreated group were HCC progression in 20 patients, liver failure in 35 and miscellaneous causes in one.

Subgroup analyses. Results of subgroup analyses according to Child-Pugh score, Milan criteria and HCC stage are shown in Table III. In all subgroup analyses, the treated group had significantly longer OS than the untreated group.

Baseline characteristics and OS in the treated and untreated groups after propensity score matching. Baseline characteristics in the two groups (treated group: n=55, untreated group: n=55) after propensity score matching are demonstrated in Table IV. For all analyzed variables, no significant differences were observed, although the AST value in the treated group tended to be lower than that in the untreated group ($p=0.055$). The MST in the treated group (MST=0.95 years, 95% CI=0.82-1.08 years) was significantly longer than that in the untreated group (MST=0.17 years, 95% CI=0.05-0.39 years) ($p=0.010$) (Figure 2).

Subgroup analyses after propensity score matching. Results of subgroup analyses after propensity score matching according to Child-Pugh score, Milan criteria and HCC stage are shown in Table V. In patients with Child-Pugh score 10 or 11 ($p=0.032$) and HCC stage III or IV ($p=0.011$), the treated group had significantly longer OS than the untreated group.

Comparison of OS of patients treated with transcatheter arterial therapies and untreated patients. The MST in the group treated with transcatheter arterial therapies (n=69,

Table II. *Univariate and multivariate analyses of factors linked to overall survival.*

Variable	Univariate analysis		Multivariate analysis		
	n	*p*-Value[a]	HR	95%CI	*p*-Value[b]
Age ≥65 years (yes/no)	91/91	0.365			
Gender (male/female)	118/64	0.105			
Treatment for HCC (yes/no)	113/69	<0.001	1.881	1.239-2.855	0.003
Child-Pugh score (10, 11/12, 13, 14, 15)	150/32	0.002	2.122	1.247-3.611	0.006
HCC stage I, II/III, IV	69/113	<0.001	1.259	0.578-2.744	0.562
Milan criteria (within/without)	79/103	<0.001	1.195	0.506-2.822	0.684
Maximum tumor size >3 cm (yes/no/unknown)	92/85/5	<0.001	0.907	0.530-1.553	0.722
AST>80 IU/l (yes/no)	91/91	0.003	1.164	0.768-1.762	0.474
ALT≥45 IU/l (yes/no)	90/92	0.068			
Platelet ≥9×10^4/mm^3 (yes/no/unknown)	91/90/1	0.098			
AFP≥100 ng/ml (yes/no/unknown)	100/80/2	0.001	1.887	1.223-2.912	0.004
DCP≥300 mAU/ml (yes/no/unknown)	80/70/32	<0.001	2.341	1.455-3.766	<0.001

HCC: Hepatocellular carcinoma, AST: aspartate aminotransferase, ALT: alanine aminotransferase, AFP: alpha-fetoprotein, DCP: des-γ-carboxy prothrombin, HR: hazard ratio, CI: confidence interval, a: log-rank test, b: Cox proportional hazard model.

MST=0.86 years, 95% CI=0.52-1.20 years) was significantly longer than that in the untreated group (p=0.001).

We further compared OS between patients treated with transcatheter arterial therapies and those untreated using propensity score matching (n=46 in both groups). The number of patients with Child-Pugh score 10/11/12/13/14, stage I or II HCC and HCC within Milan criteria in each group was similar. The MST in the group treated with transcatheter arterial therapies (MST=0.63 years, 95% CI=0.36-0.90 years) was significantly longer than that in the untreated group (MST=0.22 years, 95% CI= 0.03-0.41 years) (p=0.044).

Comparison of OS in patients treated with ablative therapies and untreated patients. The MST in the group treated with ablative therapies (n=43, MST= 1.89 years, 95% CI= 1.42-2.36 years) was significantly longer than that in the untreated group (p<0.001).

We further compared OS between patients treated with ablative therapies and those untreated using propensity score matching (n=20 in both groups). The number of patients with Child-Pugh score 10/11/12/13/14, stage I or II HCC and HCC within Milan criteria in each group was similar. The MST in the group treated with ablative therapies (MST=1.77 years, 95% CI=1.47-2.07 years) was significantly longer than that in the untreated group (MST=0.24 years, 95% CI=0.0-0.61 years; p=0.020).

Table III. *Subgroup analysis in terms of overall survival.*

	N	MST (years)	95% CI (years)	*p*-Value[a]
Child-Pugh score 10 or 11				
Treated group	99	1.21	0.87-1.55	<0.001
Untreated group	51	0.21	0.08-0.40	
Child-Pugh score 12, 13, 14 or 15				
Treated group	14	0.78	0.17-1.39	0.046
Untreated group	18	0.21	0.07-0.23	
Within Milan criteria				
Treated group	61	1.73	1.21-2.25	0.004
Untreated group	18	0.64	0.17-1.11	
Without Milan criteria				
Treated group	52	0.88	0.54-1.23	0.003
Untreated group	51	0.17	0.11-0.23	
HCC stage I or II				
Treated group	51	1.89	1.53-2.25	0.007
Untreated group	18	0.64	0.06-1.22	
HCC stage III or IV				
Treated group	62	0.89	0.69-1.09	<0.001
Untreated group	51	0.17	0.11-0.24	

HCC: Hepatocellular carcinoma, MST: median survival time, CI: confidence interval, a: log-rank test.

Discussion

As mentioned earlier, for patients with HCC with Child-Pugh C cirrhosis, most current HCC guidelines recommend liver transplantation for patients within Milan criteria and best supportive care for patients without Milan criteria (1, 4, 11, 12). Thus, few studies examined the treatment outcome in HCC patients with Child-Pugh C cirrhosis treated with nontransplant therapies (13, 22, 23). Hence, we conducted this comparative study and furthermore carried out propensity score matching analysis since selection bias might be present due to the retrospective nature of the current study.

Table IV. *Baseline characteristics of the treated group and the untreated group after propensity score matching. Data are expressed as number or mean ± standard deviation.*

Variable	Treated group (n=55)	Untreated group (n=55)	*p*-Value
Age (years)	64.3±8.5	65.9±9.7	0.454[a]
Gender, male/female	36/19	36/19	>0.999[b]
Cause of liver disease			
B/C/B and C/non B non C/unknown	8/32/3/11/1	12/33/1/9/0	0.601[b]
HCC stage			
Stage I or II / III or IV	15/40	14/41	>0.999[b]
Milan criteria			
Within/without	15/40	15/40	>0.999[b]
Maximum tumor size (cm)	4.6±2.8	5.7±3.8	0.103[a]
Child-Pugh score			
10/11/12/13/14/15	26/17/7/3/2/0	26/16/7/3/3/0	>0.999[b]
AST (IU/l)	96.3±58.8	137.3±143.4	0.055[a]
ALT (IU/l)	49.7±34.4	73.6±109.0	0.125[a]
Platelets ($\times 10^4/mm^3$)	10.7±7.2	12.9±10.0	0.109[a]
AFP (ng/ml)	27977±82313	11094±38601	0.176[a]
DCP (mAU/ml)	10640±28868	23874±48552	0.118[a]

HCC: Hepatocellular carcinoma, AST: aspartate aminotransferase, ALT: alanine aminotransferase, AFP: alpha-fetoprotein, DCP: *des*-γ-carboxy prothrombin, a: unpaired *t*-test, b: Fisher's exact test.

Table V. *Subgroup analysis in terms of overall survival after propensity score matching.*

	N	MST (years)	95% CI (years)	*p*-Value[a]
Child-Pugh score 10 or 11				
Treated group	43	0.98	0.78-1.18	0.032
Untreated group	42	0.32	0.0-0.65	
Child-Pugh score 12, 13, 14 or 15				
Treated group	12	0.55	0.01-1.09	0.249
Untreated group	13	0.15	0.0-0.31	
Within Milan criteria				
Treated group	15	1.77	0.59-2.95	0.078
Untreated group	15	0.64	0.0-1.31	
Without Milan criteria				
Treated group	40	0.82	0.48-1.16	0.060
Untreated group	40	0.21	0.10-0.32	
HCC stage I or II				
Treated group	15	1.38	0.15-2.61	0.226
Untreated group	14	0.64	0.0-1.31	
HCC stage III or IV				
Treated group	40	0.86	0.53-1.19	0.011
Untreated group	41	0.21	0.10-0.32	

HCC: Hepatocellular carcinoma, MST: median survival time, CI: confidence interval, a: log-rank test.

In our analyses, significantly better survival was shown in the treated group than in the untreated group for all cases and in all subgroup analyses, the treated group obtained significantly better survival than the untreated group. In addition, after propensity score matching, the treated group demonstrated significantly better survival than the untreated group and our multivariate analysis showed that treatment for HCC is an independent predictor linked to OS. These results suggest that nontransplant therapies can be treatment options even in patients with HCC with Child-Pugh C cirrhosis.

It is of interest that patients with stage III or IV HCC in the treated group demonstrated significantly better survival than those in the untreated group, and patients with HCC treated with transcatheter arterial therapies had significantly better prognosis than those in the untreated group even after propensity score matching. In our country, in patients with advanced-stage HCC, transcatheter arterial therapies such as TACE are selected as first-line therapy in general (10). Our results may be attributed to the frequent use and technical improvements of superselective transareterial therapies even in patients with multiple HCC tumors in Japan (10). Nouso, *et al.* reported that selective use of TACE in patients with HCC with Child-Pugh C cirrhosis provides survival benefit (22). On the other hand, in subgroup analyses in the propensity score matched cohort, no significant difference was observed in patients with Child-Pugh score 12-15 and those with HCC stage I or II in terms of OS. In these populations, liver function-related factors rather than tumor-related factors may be associated with OS.

Pretreatment DCP level was the strongest predictor associated with OS in the multivariate analysis. Kobayashi, *et al.* reported that high DCP levels reflect the biological aggressiveness and progression of HCC tumors (24). Even in patients with HCC with Child-Pugh C cirrhosis, tumor aggressiveness may be linked to poorer survival.

There are several limitations to the current study. Firstly, this is a retrospective comparative study. Secondly, in subgroups, the number of patients was small for statistical analysis. Thirdly, our study cohorts had heterogeneous patient populations with various clinical stages of HCC. Hence, a further larger prospective study is needed. However, our study results demonstrated that nontransplant therapies for patients with HCC with Child-Pugh C cirrhosis may improve prognosis.

In conclusion, in HCC with Child-Pugh C cirrhosis, patients treated with nontransplant therapies might have longer survival than untreated patients. Nontransplant therapies for HCC should not be withdrawn based solely on liver function.

Conflicts of Interest

The Authors have not received any financial support for this study and have no conflicts of interest to declare.

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Clinical significance of early interventional therapy of branched-chain amino acid granules in patients with hepatocellular carcinoma: Propensity score matching analysis

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Abstract. We examined whether supplementation of branched-chain amino acid (BCAA) granules in an early stage of underlying liver disease (pretreatment serum albumin levels ≥3.6 g/dl) can improve overall survival (OS) after therapy for hepatocellular carcinoma (HCC) using propensity score matching analysis. We compared OS between patients treated with BCAA granules and control group patients in two propensity score matched cohorts (cohort 1: pretreatment serum albumin levels ≥3.6 g/dl and <4.0 g/dl, 111 pairs; cohort 2: pretreatment serum albumin levels ≥4.0 g/dl, 61 pairs). We also performed subgroup analyses according to HCC stage. In cohort 1 patients, the OS rate in the BCAA group (median follow-up period, 2.9 years) tended to be higher compared to that in the control group (median follow-up period, 2.6 years) (1- and 3-year OS rates; 97.2 and 75.5% in the BCAA group and 87.2 and 64.5% in the control group, P=0.072), whereas in cohort 2 patients, the difference in the two groups did not reach significance in terms of OS [1- and 3-year OS rates; 83.2 and 60.7% in the BCAA group (median follow-up period, 2.3 years) and 91.8 and 66.0% in the control group (median follow-up period, 2.9 years), P=0.871]. In subgroup analyses, in cohort 1, in patients with HCC stage III or IV, the OS rate in the BCAA group (n=37) was significantly higher compared to that in the control group (n=34) (P=0.017). In other subgroup analyses, no significant difference in the two groups was found in terms of OS. In conclusion, early interventional therapies using BCAA granules may be effective in some selected HCC patients.

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Key words: hepatocellular carcinoma, branched-chain amino acid granules, early intervention, survival, propensity score matching

Introduction

Hepatocellular carcinoma (HCC) is the most common carcinoma worldwide (1). Unlike most solid cancers, both the incidence and mortality rate for HCC patients are expected to increase substantially in many countries over the next 20 years, mostly as a result of infection with hepatitis C virus (HCV), in Japan, however, the incidence of non-B and non-C HCC has recently tended to increase (2,3). Treatment methods for HCC vary depending on the disease stage and liver function, and they include surgical resection (SR), liver transplantation, radiofrequency thermal ablation (RFA), percutaneous ethanol injection therapy, transcatheter arterial chemotherapy with or without embolization, systemic treatment with molecular-targeted therapy (MTT) such as sorafenib therapy and radiation therapy (RT) (4-7). HCC also carries a considerable risk of tumor recurrence even when curative treatment was performed at the initial therapy, with the tumor characteristics and any underlying liver disease important predictive factors affecting the risk of HCC recurrence (1,2,4,6).

Branched-chain amino acids (BCAAs) are a group of essential amino acids comprising valine, leucine, and isoleucine. A low plasma level ratio of BCAAs to aromatic amino acids suggests liver cirrhosis (LC) physiologically, and BCAA supplementation was originally developed in order to normalize the patient's amino acid profile and nutritional status (8-11). Most HCC patients have underlying various stages of LC including compensated or decompensated stages. LC patients with decreased plasma BCAA level can develop protein-energy malnutrition (PEM) with increased catabolism (12). PEM is associated with a high morbidity and mortality due to an increased risk of life-threatening complications, resulting in poor clinical outcome and deteriorated quality of life (QOL) (9). PEM in LC patients is already observed in the compensated phase with serum albumin level, which is a useful indicator of liver functional reserve, ≥3.6 g/dl (13).

Supplementation with BCAA for the treatment of patients with liver disorder has been attracting attention. BCAA has a variety of pharmacological effects. BCAA treatment can correct malnutrition associated with LC (14,15) and long-term nutritional BCAA supplementation may be effective for increasing plasma BCAA levels, albumin synthesis and prevention of hepatic failure while it also improves surrogate markers in patients with advanced LC (16,17). On the other hand, dietary supplementation alone does not affect plasma BCAA levels in patients with LC (18).

BCAA granules (Livact; Ajinomoto Pharmaceutical, Tokyo, Japan) have been approved for its use in LC patients since 1996 in Japan, and BCAA granules are generally administered in LC patients with a serum albumin level ≤3.5 g/dl. Clinical evidence regarding the effect of this therapy is being accumulated (19-23). Thus, the 2008 Japanese guidelines for the treatment of patients with chronic liver diseases recommend that treatment with BCAA granules should be performed for decompensated LC with a serum albumin level ≤3.5 g/dl (19). In Japan, BCAA granules are widely used in clinical practice without serious adverse effects. In addition, we have previously demonstrated that BCAA treatment may improve OS and recurrence-free survival after RFA in patients with HCV-related HCC ≤3 cm in diameter with ≤3 nodules and a serum albumin level before RFA ≤3.5 g/dl (24). On the other hand, several investigators reported that for patients with chronic liver diseases including LC, it might be beneficial to initiate BCAA therapy in the compensatory stage or even earlier, which means 'early intervention using BCAA therapy', rather than starting BCAA therapy in the decompensatory stage (25-27). Thus, BCAA supplementation may be effective in improving clinical outcome in cirrhotic patients regardless of disease stage.

However, to our knowledge, whether this early intervention using BCAA granules in patients with HCC can contribute to prolongation of survival remains unclear. The objectives of the present study were to examine whether supplementation of BCAA granules in an early stage of underlying liver disease (pretreatment serum albumin level ≥3.6 g/dl) can improve overall survival (OS) after HCC therapy. For reducing selection biases, we used propensity score matching analysis.

Patients and methods

Patients. A total of 1,134 consecutive treatment-naïve patients diagnosed with HCC with pretreatment serum albumin level ≥3.6 g/dl were admitted to the Department of Gastroenterology and Hepatology, Osaka Red Cross Hospital, Japan, between 2004 and 2013. We divided these patients into two groups according to pretreatment serum albumin level (cut-off serum albumin level, 4.0 g/dl). There were 470 patients with pretreatment serum albumin level ≥3.6 g/dl and <4.0 g/dl at the initial treatment for HCC. Of these, BCAA granules were prescribed in 120 patients at the initial therapy for HCC and the remaining 350 patients did not receive such therapy at the initial therapy for HCC. Since this study was a retrospective observational study, covariate adjustment using the propensity score was performed. One hundred and eleven pairs were thus selected for analysis in this cohort (cohort 1) (Fig. 1). Similarly, there were 664 patients with pretreatment serum albumin level ≥4.0 g/dl at the initial treatment for HCC. Of these, BCAA granules were prescribed in 63 patients at the initial therapy for HCC and the remaining 601 patients did not receive such therapy at the initial therapy for HCC. After using the propensity score matching, 61 pairs were selected for analysis in this cohort (cohort 2) (Fig. 1). We compared the OS rate between the BCAA group and the control group in each cohort.

Prior to therapy for HCC, written informed consent was obtained from all patients. The ethics committee of our department approved the protocol for HCC therapy. The present study comprised a retrospective analysis of patients' medical records in our database and all treatments were performed in an open-label manner.

Diagnosis of HCC and HCC therapy. HCC was diagnosed based on the results from abdominal ultrasound and dynamic computed tomography (CT) scan (hyperattenuation during the arterial phase in the entire or part of the tumor, and hypoattenuation in the portal-venous phase) and/or magnetic resonance imaging mainly as recommended by the American Association for the Study of Liver Diseases (28). Arterial and portal phase dynamic CT images were obtained ~30 and 120 sec after injection of contrast material. For all patients, abdominal angiography combined with CT (angio-CT) was performed before therapy for HCC after obtaining informed consent from them for performing abdominal angiography. This was performed based on the fact that this technique was useful for detecting small satellite nodules as reported by Yamasaki *et al* (29). Then, we confirmed HCC using CT during hepatic arteriography (CTHA) and CT during arterial-portography (CTAP). As for HCC therapy, the most appropriate treatment modality for each patient was selected through discussion with surgeons, hepatologists and radiologists (30,31). In the present analysis, there was no patient treated with liver transplantation and there was no treatment related death.

BCAA granule treatment. The patient's attending physician determined whether treatment with BCAA granules would be performed in individual patients considering their intent to receive the treatment after providing sufficient information regarding BCAA treatment to them. In the BCAA group, BCAA granules, containing 952 mg of L-isoleucine, 1,904 mg of L-leucine and 1,144 mg of L-valine per sachet, were orally administered to subjects at a dose of one sachet three times daily after meals ≥1 month after initial therapy for HCC. We confirmed in our database that patients in the BCAA group were prescribed BCAA granules regularly.

Follow-up after initial therapy for HCC. Follow-up observation consisted of regular blood tests and monitoring of tumor markers, including α-fetoprotein (AFP) and des-γ-carboxy prothrombin (DCP), which was measured using a chemiluminescent enzyme immunoassay (Lumipulse PIVKAII Eisai, Eisai, Tokyo, Japan). Dynamic CT scan was performed every 3-4 months after initial therapy for HCC. In particular, for patients in the BCAA group, we confirmed that BCAA granules were taken properly at every hospital visit. When HCC recurrence or disease progression was detected based on radiologic findings, most appropriate therapy was performed in each patient.

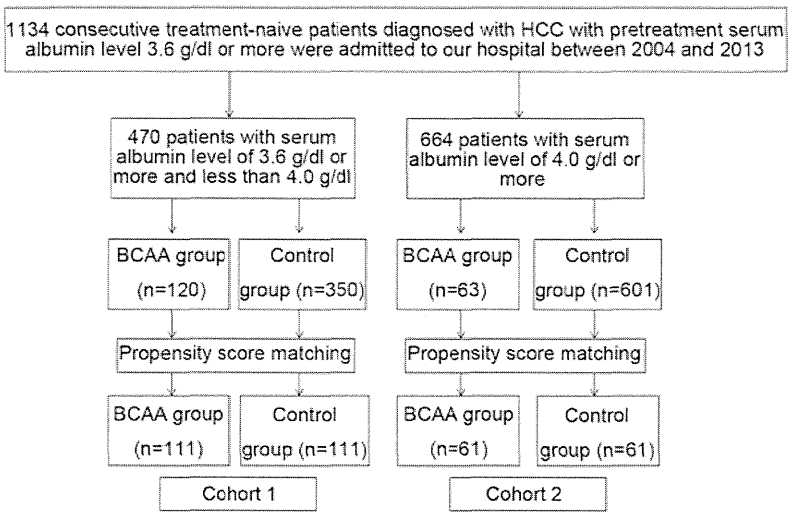

Figure 1. Study design.

Statistical analysis. The primary end-point is OS. Continuous variables were compared by unpaired t-test, and categorical variables were compared by Fisher's exact test. Data were analyzed using univariate and multivariate analyses. The cumulative OS rate was calculated by Kaplan-Meier method and tested by log-rank test. A Cox proportional hazard model was used for multivariate analyses of factors with P<0.1 in univariate analysis. These statistical methods were used to estimate the interval from each initial therapy for HCC. Data were analyzed using SPSS software (SPSS Inc., Chicago, IL, USA) for Microsoft Windows. Data are expressed as mean ± standard deviation. A P-value <0.05 was considered to be statistically significant.

Propensity score analysis. To compare the OS between BCAA group patients and control group patients, a propensity score model was used with an attempt to reduce potential biases in survival analysis (32,33). Possible variables associated with long-term survival of HCC patients, including age, sex, HCC stage, maximum tumor size, cause of liver disease, serum albumin level, aspartate aminotransferase (AST) value and alanine aminotransferase (ALT) value were included comprehensively for propensity score generation. With these selected variables, a logistic regression was applied to generate a continuous propensity score from 0 to 1. One-to-one matches between BCAA group patients and control group patients were introduced into the subsequent analysis.

Results

Patient demographic characteristics and survival (cohort 1). Baseline demographic characteristics of patients in cohort 1 are shown in Table I. There were 111 patients in the BCAA group and 111 patients in the control group, respectively. In terms of baseline demographic characteristics, no significant differences were noted between the BCAA group and the control group, showing that balance of baseline characteristics in the two groups was obtained in the matched sample. As an initial therapy for HCC, in the BCAA group, SR was performed in 35 patients, percutaneous ablative therapies in 52, transcatheter arterial chemotherapy with or without embolization in 22, MTT in one and RT in one, whereas in the control group, SR was performed in 23 patients, percutaneous ablative therapies in 64, transcatheter arterial chemotherapy with or without embolization in 21 and MTT in three (P=0.201).

The median follow-up period was 2.9 years (range, 0.5-7.0 years) in the BCAA group and 2.6 years (range, 0.1-7.6 years) in the control group. Thirty-nine patients (35.2%) in the BCAA group died during the follow-up period. The causes of death were HCC progression (24 patients), liver failure (12 patients) and miscellaneous (3 patients). Sixty patients (54.1%) in the control group died during the follow-up period. The causes of death were HCC progression (40 patients), liver failure (14 patients) and miscellaneous (6 patients).

The 1-, 3- and 5-year OS rates after each initial therapy for HCC were 97.2, 75.5 and 56.3%, respectively, in the BCAA group and 87.2, 64.5 and 38.8%, respectively, in the control group (P=0.072) (Fig. 2), indicating that the OS rate in the BCAA group tended to be higher compared to that in the control group.

Univariate and multivariate analysis of factors contributing to OS (cohort 1). In patients with pretreatment serum albumin level of ≥3.6 and <4.0 g/dl, using univariate analyses of factors contributing to OS, HCC stage (P<0.001), maximum tumor size ≥2.5 cm (P=0.004), AST ≥50 IU/l (P=0.004), ALT ≥40 IU/l (P=0.018), alkaline phosphatase (ALP) ≥330 IU/l (P=0.013), γ glutamyl transpeptidase (GGT) ≥70 IU/l (P=0.005), AFP ≥100 ng/ml (P=0.001) and DCP ≥100 mAU/ml (P<0.001) were found to be significant factors (Table II). The multivariate analyses involving nine factors with P<0.1 in the univariate analysis showed that only HCC stage was a significant independent predictor linked to OS (P=0.001). The hazard ratios (HRs), 95% confidence interval (CI) and P-value for nine factors are detailed in Table II.

Patient demographic characteristics and survival (cohort 2). Baseline demographic characteristics of patients in cohort 2 are shown in Table III. They included 61 patients in the BCAA group and 61 patients in the control group, respectively. In

Table I. Baseline characteristics between the BCAA group and the control group in HCC patients with pretreatment serum albumin level ≥3.6g/dl and <4.0 g/dl after propensity score matching.

Variables	BCAA group (n=111)	Control group (n=111)	P-value
Age (years)	67.8±9.3	69.8±9.6	0.144[a]
Gender, male/female	77/34	75/36	0.885[b]
Causes of liver disease			
B/C/non-B non-C/B and C	7/71/32/1	9/79/23/0	0.348[b]
HCC stage I/II/III/IV	27/47/31/6	26/51/23/11	0.417[b]
Maximum tumor size (cm)	3.2±2.3	3.4±2.7	0.665[a]
Initial therapy for HCC			
SR/ablation/transcatheter arterial chemotherapy/MTT/RT	35/52/22/1/1	23/64/21/3/0	0.201[b]
AST (IU/l)	57.1±28.3	63.8±41.6	0.170[a]
ALT (IU/l)	48.5±31.5	56.4±51.2	0.164[a]
ALP (IU/l)	383.8±165.3	406.5±331.5	0.520[a]
GGT (IU/l)	96.9±86.8	119.3±152.5	0.158[a]
Serum albumin (g/dl)	3.8±0.1	3.7±0.1	0.684[a]
Total bilirubin (mg/dl)	1.0±0.5	1.0±0.6	0.419[a]
Prothrombin time (%)	81.8±12.9	84.1±13.9	0.180[a]
Platelets (x10^4/mm^3)	10.8±5.8	11.4±4.1	0.194[a]
AFP (ng/ml)	1,548±12,111	2,918±25,034	0.604[a]
DCP (mAU/ml)[c]	7,570±49,443	3,372±12,614	0.387[a]

Data are expressed as number or mean ± standard deviation. BCAA, branched-chain amino acid; HCC, hepatocellular carcinoma; MTT, molecular targeting therapy; RT, radiation therapy; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, γ glutamyl transpeptidase; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin. [a]Unpaired t-test; [b]Fisher's exact test; [c]missing data, n=1.

Table II. Univariate and multivariate analysis of factors contributing to overall survival in HCC patients with pretreatment serum albumin level ≥3.6 and <4.0 g/dl (cohort 1).

Variables	n	Univariate analysis P-value[a]	Multivariate analysis Hazard ratio (95% CI)	Multivariate analysis P-value[b]
Gender, male vs. female	152/70	0.558		
Age (years), ≥70 vs. <70	111/111	0.739		
BCAA vs. control	111/111	0.072	1.372 (0.887-2.123)	0.155
HCC stage I or II vs. III or IV	151/71	<0.001	0.376 (0.214-0.659)	0.001
Maximum tumor size (cm), ≥2.5 vs. <2.5	105/117	0.004	0.964 (0.575-1.616)	0.889
Cause of liver disease, viral vs. non-viral	167/55	0.523		
AST (IU/l), ≥50 vs. <50	116/106	0.004	0.731 (0.387-1.380)	0.333
ALT (IU/l), ≥40 vs. <40	111/111	0.018	0.962 (0.520-1.780)	0.903
ALP (IU/l), ≥330 vs. <330	116/106	0.013	0.804 (0.515-1.254)	0.336
GGT (IU/l), ≥70 vs. <70	111/111	0.005	0.884 (0.563-1.389)	0.593
Serum albumin level (g/dl), ≥3.8 vs. <3.8	108/114	0.374		
Total bilirubin (mg/dl), ≥1.0 vs. <1.0	90/132	0.117		
Platelet count (x10^4/mm^3), ≥10 vs. <10	106/116	0.472		
Prothrombin time (%), ≥70 vs. <70	187/35	0.687		
Serum AFP (ng/ml), ≥100 vs. <100	62/160	0.001	0.788 (0.500-1.242)	0.305
DCP (mAU/ml), ≥100 vs. <100[c]	78/143	<0.001	0.767 (0.458-1.282)	0.311

BCAA, branched-chain amino acid; HCC, hepatocellular carcinoma; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, γ glutamyl transpeptidase; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; CI, confidence interval. [a]Log-rank test; [b]Cox proportional hazard model; [c]missing data, n=1.

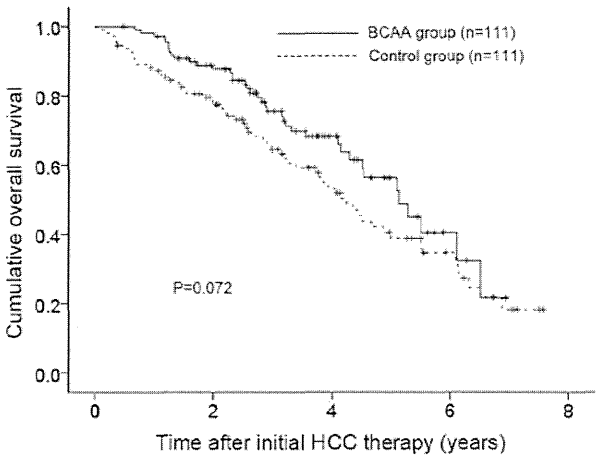

Figure 2. Cumulative overall survival in patients with pretreatment serum albumin level ≥3.6 and <4.0 g/dl (cohort 1). The 1-, 3- and 5-year OS rates after each therapy for HCC were 97.2, 75.5 and 56.3%, respectively, in the BCAA group (n=111) and 87.2, 64.5 and 38.8%, respectively, in the control group (n=111) (P=0.072).

terms of baseline demographic characteristics, no significant differences were noted between the BCAA group and the control group, demonstrating that balance of baseline characteristics in the two groups was obtained in the matched sample. As an initial therapy for HCC, in the BCAA group, SR was performed in 31 patients, percutaneous ablative therapies in 15 and transcatheter arterial chemotherapy with or without embolization in 15, whereas in the control group, SR was performed in 20 patients, percutaneous ablative therapies in 19, transcatheter arterial chemotherapy with or without embolization in 21 and MTT in one (P=0.160).

The median follow-up period was 2.3 years (range, 0.2-7.8 years) in the BCAA group and 2.9 years (range, 0.3-8.4 years) in the control group. Twenty-four patients (39.3%) in the BCAA group died during the follow-up period. The causes of death were HCC progression (22 patients) and liver failure (2 patients). Twenty-eight patients (45.9%) in the control group died during the follow-up period. The causes of death were HCC progression (24 patients), liver failure (2 patients) and miscellaneous (2 patients).

The 1-, 3- and 5-year OS rates after each initial therapy for HCC were 83.2, 60.7 and 54.0%, respectively, in the BCAA group and 91.8, 66.0 and 44.5%, respectively, in the control group (P=0.871) (Fig. 3).

Univariate and multivariate analysis of factors contributing to OS (cohort 2). In patients with serum albumin level of ≥4.0 g/dl, using univariate analyses of factors contributing to OS, HCC stage (P<0.001), maximum tumor size ≥4.0 cm (P<0.001), AST ≥50 IU/l (P=0.024), ALP ≥300 IU/l (P<0.001), GGT ≥70 IU/l (P=0.009), total bilirubin ≥1.0 mg/dl (P=0.045), AFP ≥100 ng/ml (P=0.013) and DCP ≥100 mAU/ml (P<0.001) were found

Table III. Baseline characteristics between the BCAA group and the control group in HCC patients with pretreatment serum albumin level ≥4.0 g/dl after propensity score matching.

Variables	BCAA group (n=61)	Control group (n=61)	P-value
Age (years)	69.8±10.0	69.9±9.7	0.985[a]
Gender, male/female	43/18	42/19	>0.999[b]
Causes of liver disease			
B/C/non-B non-C/B and C	10/29/21/1	8/31/21/1	0.954[b]
HCC stage I/II/III/IV	6/26/20/9	7/27/23/4	0.555[b]
Maximum tumor size (cm)	4.9±2.9	4.7±3.3	0.686[a]
Initial therapy for HCC			
SR/ablation/transcatheter arterial chemotherapy/MMT	31/15/15/0	20/19/21/1	0.160[b]
AST (IU/l)	67.0±54.7	59.3±36.4	0.183[a]
ALT (IU/l)	55.0±37.2	54.1±47.4	0.905[a]
ALP (IU/l)	365.5±204.7	320.6±131.5	0.139[a]
GGT (IU/l)	128.9±151.7	133.0±145.3	0.879[a]
Serum albumin (g/dl)	4.2±0.2	4.3±0.2	0.344[a]
Total bilirubin (mg/dl)	1.0±0.4	0.8±0.3	0.167[a]
Prothrombin time (%)	89.2±11.8	93.4±15.1	0.209[a]
Platelets ($x10^4/mm^3$)	14.7±8.7	14.1±3.9	0.627[a]
AFP (ng/ml)	15,065±82,251	3,018±18,567	0.267[a]
DCP (mAU/m)[c]	7,011±28,784	7,926±24,508	0.853[a]

Data are expressed as number or mean ± standard deviation. BCAA, branched-chain amino acid; HCC, hepatocellular carcinoma; MTT, molecular targeting therapy; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, γ glutamyl transpeptidase; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin. [a]Unpaired t-test; [b]Fisher's exact test; [c]missing data, n=3.

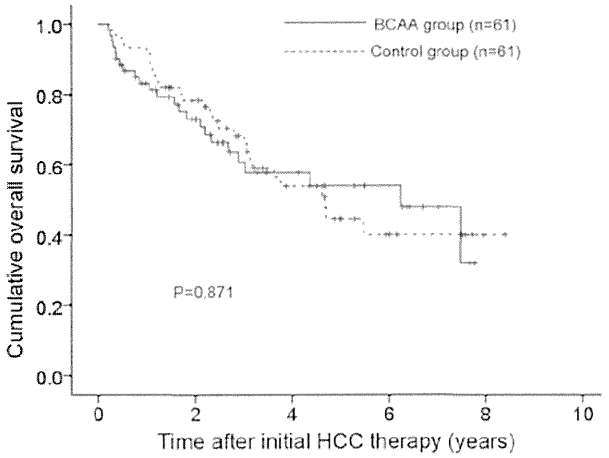

Figure 3. Cumulative overall survival in patients with pretreatment serum albumin level ≥4.0 g/dl (cohort 2). The 1-, 3- and 5-year OS rates after each therapy for HCC were 83.2, 60.7 and 54.0%, respectively, in the BCAA group (n=61) and 91.8, 66.0 and 44.5%, respectively, in the control group (n=61) (P=0.871).

to be significant factors (Table IV). The multivariate analyses involving nine factors with P<0.1 in the univariate analysis showed that HCC stage (P=0.019), ALP ≥300 IU/l (P=0.023) and AFP ≥100 ng/ml (P=0.019) were significant independent predictors linked to OS. The HRs, 95% CI and P-value for nine factors are detailed in Table IV.

Subgroup analysis according to HCC stage. Since HCC stage was an independent predictor associated with OS in both cohort 1 and 2, we further performed subgroup analyses according to HCC stage. In cohort 1 patients, there were 74 patients with HCC stage I or II in the BCAA group and 77 patients with HCC stage I or II in the control group. In terms of OS, no significant difference was observed in the two groups (P=0.353) (Fig. 4A). In cohort 1 patients, there were 37 patients with HCC stage III or IV in the BCAA group and 34 patients with HCC stage III or IV in the control group. In terms of OS, the difference in the two groups reached significance (P=0.017) (Fig. 4B). On the other hand, cohort 2 patients included 32 and 29 patients with HCC stage I or II and III or IV in the BCAA group and 34 and 27 patients with HCC stage I or II and III or IV in the control group. Regardless of HCC stage, no significant difference was found in terms of OS (P=0.785 for HCC stage I or II and P=0.572 for HCC stage III or IV) (Fig. 5).

Discussion

BCAA granules have a variety of pharmacological effects. Kawaguchi *et al* (8,35) and Kawaguchi and Sata (34) showed that BCAA granules can improve albumin synthesis, insulin resistance, immune function and patients' QOL while they can reduce liver related complications and occurrence of HCC. In addition, BCAA supplementation can help in the management of HCC patients since most HCC patients have underlying LC

Table IV. Univariate and multivariate analysis of factors contributing to overall survival in HCC patients with pretreatment serum albumin level ≥4.0 g/dl (cohort 2).

Variables	n	Univariate analysis P-value[a]	Multivariate analysis Hazard ratio (95% CI)	Multivariate analysis P-value[b]
Gender, male vs. female	85/37	0.422		
Age (years), ≥72 vs. <72	60/62	0.880		
BCAA vs. control	61/61	0.871		
HCC stage I or II vs. III or IV	66/56	<0.001	0.437 (0.219-0.872)	0.019
Maximum tumor size (cm), ≥4.0 vs. <4.0	58/64	<0.001	0.610 (0.307-1.213)	0.159
Cause of liver disease, viral vs. non-viral	80/42	0.517		
AST (IU/l), ≥50 vs. <50	61/61	0.024	0.679 (0.307-1.502)	0.339
ALT (IU/l), ≥40 vs. <40	65/57	0.058	0.486 (0.210-1.127)	0.093
ALP (IU/l), ≥300 vs. <300	62/60	<0.001	0.466 (0.241-0.902)	0.023
GGT (IU/l), ≥80 vs. <80	61/61	0.009	0.579 (0.299-1.122)	0.105
Serum albumin level (g/dl), ≥4.3 vs. <4.3	49/73	0.456		
Total bilirubin (mg/dl), ≥1.0 vs. <1.0	40/82	0.045	0.897 (0.465-1.730)	0.746
Platelet count ($x10^4/mm^3$), ≥13 vs. <13	65/57	0.372		
Prothrombin time (%), ≥80 vs. <80	102/20	0.937		
Serum AFP (ng/ml), ≥100 vs. <100	48/74	0.013	0.481 (0.260-0.889)	0.019
DCP (mAU/ml), ≥100 vs. <100[c]	73/46	<0.001	0.560 (0.250-1.254)	0.158

BCAA, branched-chain amino acid; HCC, hepatocellular carcinoma; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, γ glutamyl transpeptidase; AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; CI, confidence interval. [a]Log-rank test; [b]Cox proportional hazard model; [c]missing data, n=3.

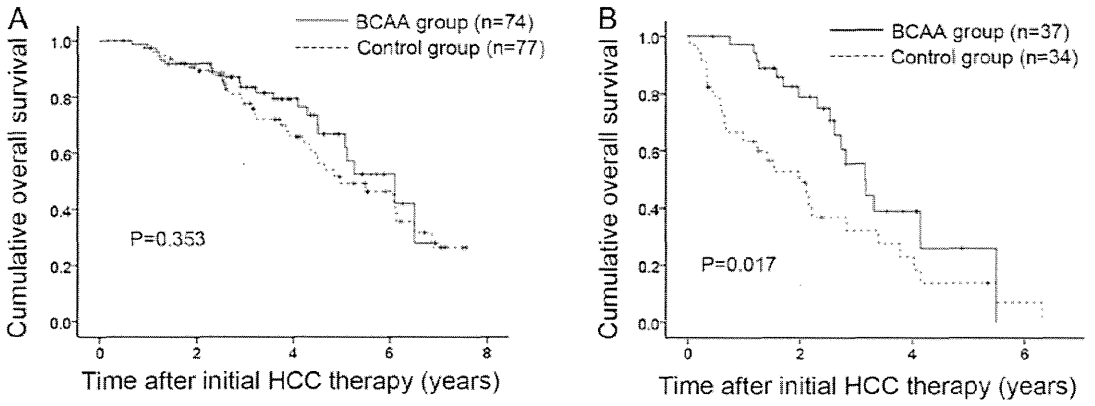

Figure 4. Subgroup analyses in patients with HCC stage I or II (n=74 in the BCAA group and n=77 in the control group) (A) and HCC stage III or IV (n=37 in the BCAA group and n=34 in the control group) (B) in cohort 1 (pretreatment serum albumin level ≥3.6 g/dl and <4.0 g/dl) in terms of OS.

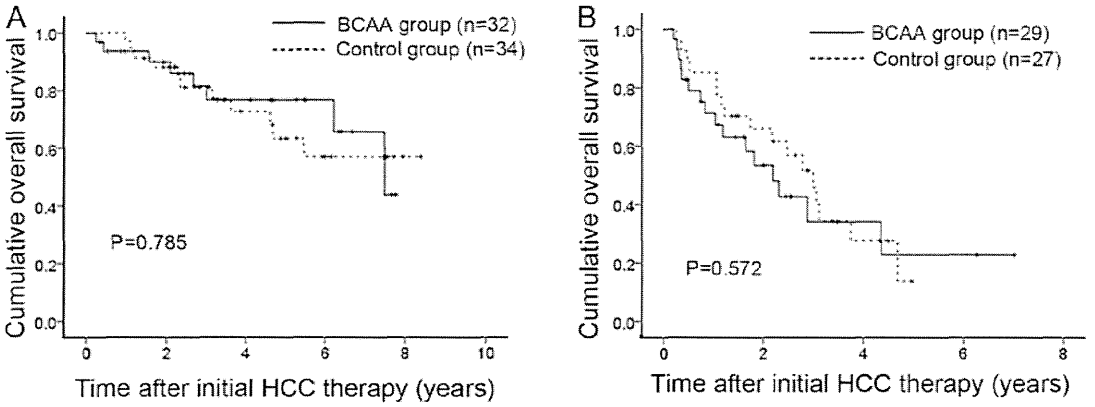

Figure 5. Subgroup analyses in patients with HCC stage I or II (n=32 in the BCAA group and n=34 in the control group) (A) and HCC stage III or IV (n=29 in the BCAA group and n=27 in the control group) (B) in cohort 2 (pretreatment serum albumin level ≥4.0 g/dl) in terms of OS.

(24,27,36,37). However, whether early interventional therapy using BCAA granules in patients with HCC can improve survival remains unclear. An interesting issue is when is the optimal timing of nutritional support such as BCAA granules in patients with HCC. Hence, we conducted these comparative studies.

In our results, in cohort 1 (patients with pretreatment serum albumin level ≥3.6 and <4.0 g/dl), the OS rate in the BCAA group tended to be higher compared to that in the control group and in subgroup analysis in patients with HCC stage III or IV, which means advanced stage of HCC, the OS rate in the BCAA group was significantly higher compared to that in the control group, although in other analyses, no significant difference in the two groups was found. These results suggest that early interventional therapy using BCAA granules can be a treatment option for some selected patients.

In general, in patients with advanced stage HCC, curative therapies are difficult to perform due to tumor characteristics. Hence, repeated therapies for HCC will be needed in these patients. However, these repeated therapies can lead to deterioration of liver functional reserve as reflected by hypoalbuminemia (24,27). BCAA supplementation actually improves hypoalbuminemia. Moreover, Kawaguchi *et al* reported that BCAA granules may suppress hepatic neovascularization and hepatocarcinogenic activity and Yoshiji *et al* demonstrated that BCAA therapy significantly suppressed glucose- and insulin-induced angiogenesis in the presence of vascular endothelial growth factor (VEGF) (8,22). Angiogenesis is a key process in tumor growth and VEGF, which stimulates angiogenesis, appears to be essential for HCC progression (8,22). Our present results may be associated with these observations. On the other hand, advanced malignancy can result in muscle wasting and systemic catabolism, with BCAA treatment having the potential to improve these poor conditions (36).

As described above, in cohort 2 patients (pretreatment serum albumin level ≥4.0 g/d), the difference in the BCAA and control groups did not reach significance in the analyses in terms of OS. Very early intervention using BCAA granules might not be beneficial for improving OS. Curative treatment at the initial therapy for HCC, close surveillance for HCC recurrence and adequate therapy for recurrence will be more important than nutritional therapy in these patients for prolonging OS.

This study included several limitations. First, this is a retrospective observational study although propensity score matching analyses were performed for reducing selection biases in this study. Second, our patient cohorts included heterogeneous patient populations with various stages of HCC

and various causes of underlying liver diseases. Third, BCAA treatment adherence in each individual, antiviral therapies such as interferon therapy for patients with HCV or nucleoside analogue therapy for those with hepatitis B virus during observation period were not included in the present analyses, leading to bias (38-40). Hence, further studies with well selected patient population will be needed. However, our study results demonstrated that early interventional therapy using BCAA granules may be effective in some selected patients.

We concluded that in HCC patients with pretreatment serum albumin level ≥3.6g/dl, early BCAA supplementation can be a treatment option for improving clinical outcome.

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